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MOTOR ENDPLATE ANALYSIS OF THE DENERVATED AND REINNERVATED ORBICULARIS OCULI MUSCLE IN THE RAT

ABSTRACT

The present study examined the histochemical characteristics of the orbicularis oculi muscle (OOM) in the rat, in order to better understand the target muscle of the blink reflex—specifically, the motor endplate distribution and number in the normal, denervated, and reinnervated OOM. Assessment of the number of endplates needed to accomplish eye closure would provide critical information in the microsurgical restoration of the blink reflex in facial paralysis. Results demonstrated a 50% increase in the number of endplates of reinnervated rats, compared to denervated animals.

Facial paralysis is a disturbing, clinical, facial-nerve disorder of varied etiology. Severe cranial infection, inflammation, developmental anomalies, or compression and erosion by tumor are some of the prevalent causes of VII nerve dysfunction. The most unsettling sequela of VII nerve paralysis is the loss of the blink reflex, leading to both functional and aesthetic deformity. The blink reflex is an important measure which has largely been under-utilized in the evaluation of patients with facial paralysis. While the literature contains some information on the blink reflex in the normal state, reports addressing its absence in the paralytic face and restoration of the blink reflex via surgical manipulation are sparse.[1]

Electrophysiologically, it has been demonstrated that the blink reflex is composed of two distinct electrical components[2]: 1) an early ipsilateral (R1) component that consists of an oligosynaptic circuit with a short latency (10 to 12 msec) and which results in subclinical eye sphincter contraction; and 2) the major component R2 (bilateral) that involves a polysynaptic circuit with a longer latency (20 to 45 msec) and is associated with eye closure.

The neuroanatomy of the blink reflex circuit consists of a sensory limb, involving corneal or cutaneous stimuli about the orbit, which are then transmitted via trigeminal nerve fibers. These afferent impulses project to the facial nerve motor nuclei, which then elicit efferent signals primarily to the orbicularis oculi muscle (OOM) via branches of the facial nerve, resulting in eye closure.[3,4] In addition, blinking involves, to a lesser degree, the inhibition of the levator palpebrae muscle and activation of the superior rectus muscle.[3]

A variety of approaches have been employed to treat the deficits associated with facial nerve paralysis. Initially, the use of cross-over procedures gained popularity, such as the accessory-to-facial and hypoglossal-to-facial transfer.[5,6] While resting tonus was restored to all facial muscle groups, the sacrifice of a functioning cranial nerve and its target muscles was required. A more recent approach involves the use of interposition cross-facial nerve grafts linking the paralyzed facial muscles to branches of the normal contralateral facial nerve.[7,8]

Gyo *et al.*[2] reported that the blink reflex test had distinct advantages over other tests in the diagnosis

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of facial palsy, because the blink reflects the status of the entire facial nerve. Recently, our laboratory has established an experimental model to study the blink reflex in the rat.[9] Specifically, facial paralysis in the rat was accomplished by unilateral transection of the VII nerve trunk and subsequent reinnervation by a cross-facial nerve graft (CFNG).

The present study examined the distribution and number of motor end-plates in the normal, denervated, and reinnervated OOM. The number of end-plates needed to accomplish eye closure is critical information that is needed in the microsurgical restoration of the blink reflex in paralysis of the facial nerve, and this datum addresses some of the histologic and pathologic questions associated with facial paralysis.

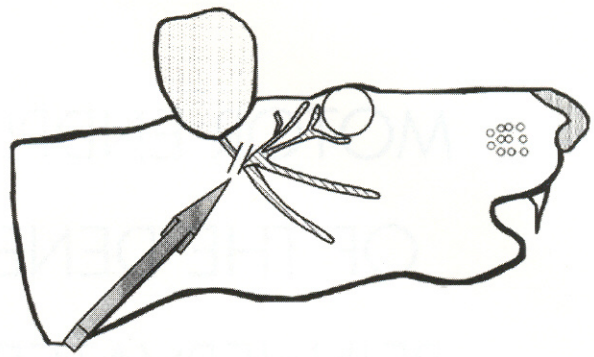

Figure 1. Diagram of Group 2 surgery. Arrow indicates right facial nerve trunk.

MATERIALS AND METHODS

Male, adult, Sprague-Dawley rats, weighing 350 to 450 g, were housed in an institutional facility and given food and water *ad libitum*. All animals were treated in accordance with the guidelines established by the Animal Use and Care Committee. The animals were divided into three groups. Group 1—The control group consisted of normal rats who had their OOM harvested and analyzed (n = 3). Group 2—The denervated group consisted of animals who had their right facial nerve transected (n = 10). Group 3—The reinnervated group received right facial nerve transection and were treated with a CFNG (n = 10). Surgical procedures were performed under general anesthesia (ketamine/xylazine i.m.), utilizing a surgical operating Zeiss microscope.

STAGE I. *Group* 2. The left facial nerve and its branches were explored and dissected via a preauricular incision. The temporal and zygomatic branches to the eye were identified by intraoperative nerve stimulation. The right facial nerve trunk was then explored and stimulated and subsequently transected, producing a complete right facial paralysis. In addition, intraoperative photos and videotapes were taken before and after the transection (Fig. 1).

Group 3. The right facial nerve trunk and its branches were explored and dissected through a preauricular incision. The temporal and/or zygomatic branches to the eye were identified, using intraoperative nerve stimulation, photographed, and videotaped. The right facial nerve trunk was then transected, producing a complete right facial paralysis (Fig. 2). The saphenous nerve was then harvested as a graft and tunneled in the subcutaneous plane over the dorsum of the cranium to the right side through the incision (see Fig. 2). Biopsies of the eye branches and both ends of the nerve graft were taken and coaptations of the left eye branch to the saphenous nerve graft performed using 11-0 sutures with a 30 micron needle.

STAGE II. *Group* 3. Three months following stage I, the right face was explored, using a preauricular incision, and the cross-facial nerve graft was coapted to the distal end of the transected eye branch, a specimen of which was taken for biopsy (see Fig. 2). Intraoperative videotapes were taken for documentation.

STAGE III. *Groups* 2 *and* 3. Three months following stage II, the coaptations of the right eye branches with the grafts were explored, and nerve stimulation performed proximal and distal to the coaptation site and in the midgraft region. The entire graft was then harvested along with the OOM for motor endplate analysis. Intraoperative videotapes were again taken for documentation.

HISTOLOGY. Harvested OOMs were frozen in a liquid nitrogen bath and stored at −80° C. To quantify endplates, the frozen orbicularis oculi muscles were positioned horizontally and sectioned in a serial fashion at 30 to 35 microns on a cryostat and stained with an acetylcholinesterase stain.[10]

BLINK REFLEX. Each animal was photographed and videotaped prior to any surgical procedure and at weekly intervals thereafter, to document the status and symmetry of both eye sphincters. The blink reflex was evaluated utilizing a custom-designed apparatus that delivered a constant 20 ml volume of air to the cornea and periorbital region at a distance of 2 cm. The air stimulus was delivered through a plastic microtubing system, connected to a volume reservoir located away from the animal. This apparatus provided an adequate stimulus for qualitative analysis of the blink reflex and was a reliable method in determining the status of the reflex.

ANALYSIS. Quantitative assessment of the endplates, morphology, and distribution were studied using the Zeiss universal light microscope. Examination of endplate distribution was performed by dividing the orbicularis oculi muscle into quadrants (Fig. 3).

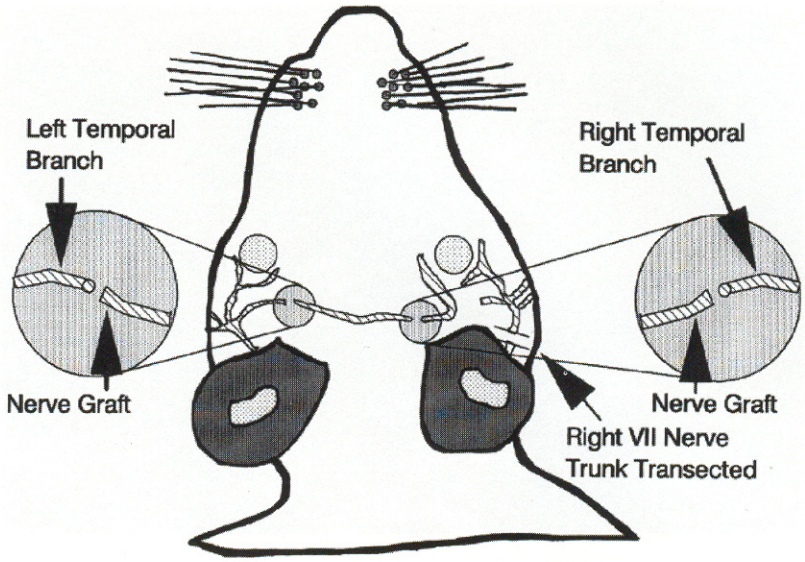

Figure 2. Diagram of Group 3 surgery.

RESULTS

Behavior Analysis. The behavioral studies have demonstrated that all animals undergoing stage I surgery exhibited complete right-facial paralysis and loss of the blink reflex. However, animals treated with the cross-facial nerve graft procedure demonstrated recovery of the blink response at 3 months (stage III). In addition, it was demonstrated that the electrophysiologic components of the rat blink paralleled those seen in humans, but the latencies were slightly shorter.[9]

Histochemistry. The OOM sections were stained with an acetylcholinesterase stain[10] and subsequently examined morphologically. Specifically, the distribution of the endplates was assessed and a quantitative analysis was carried out. An example of the motor endplates in a normal, rat-eye sphincter is illustrated in Figure 4.

Nerve-Muscle Relationship. Previous research in this laboratory has demonstrated the anatomic dissections of the facial nerve and the facial musculature in both the cadaver and the rat (Fig. 5). These studies demonstrated in the rat that the orbicularis oculi muscle is innervated by two facial nerve branches—the temporal and the zygomatic (each of these branches is bifascicular in nature). Thus, it has been feasible in our experimental model to borrow motor axons by performing selective neurectomy and coapting these to an interposition cross-facial nerve graft from the normal side, linking them to the paralyzed side. In addition, the location of the motor endplates in the OOM is predominantly lateral, as it receives its motor-nerve fibers in the superior and inferior lateral quadrants.

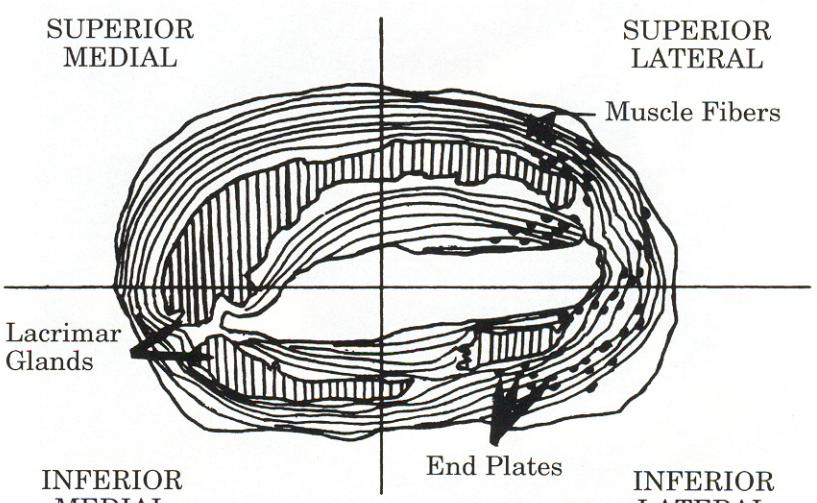

Figure 3. Diagram of rat OOM (longitudinal section, ×25).

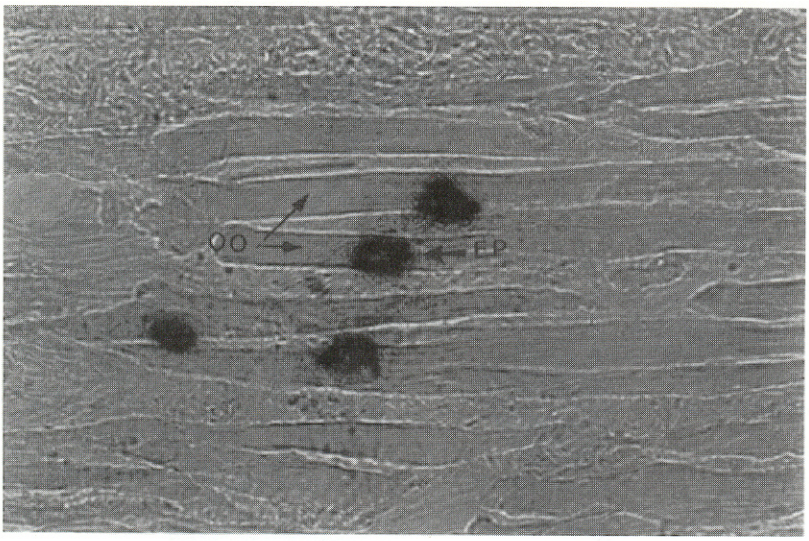

Figure 4. Longitud
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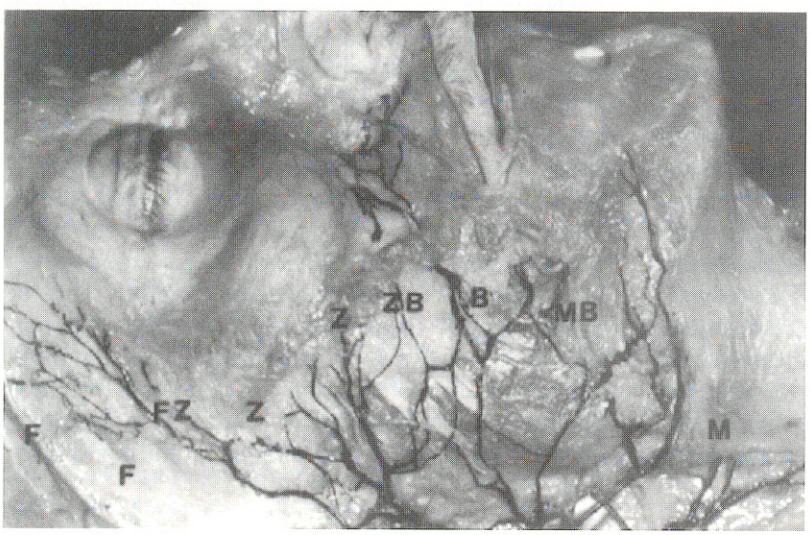

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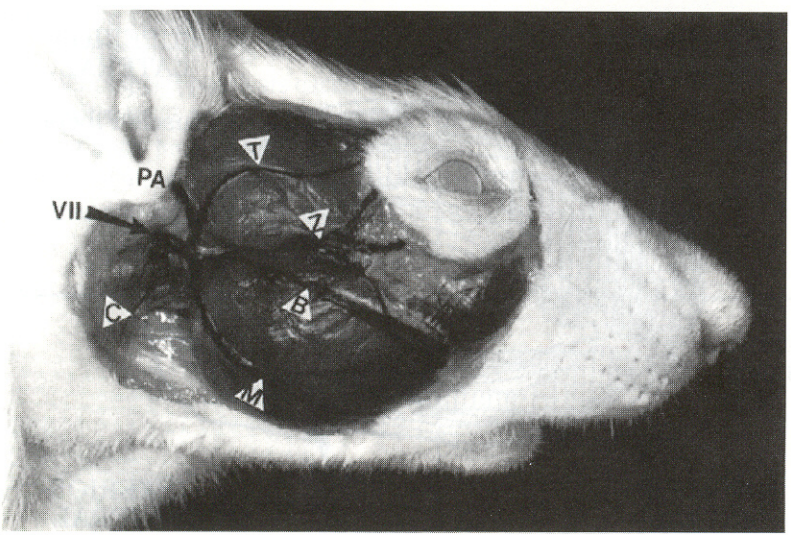

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Figure 5. A, Facial
dissection in a fresh hu
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Z = zygomatic; ZB = zyg
cal; B = buccal; MB =
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1989.) B, Facial nerve r
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temporal; Z = zygomatic
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QUANTITATIVE ANALYSIS. Assessment of the number of endplates between groups, using a one-way ANOVA analysis showed a highly significant effect ($p < 0.001$), specifically, a significant decrease ($p < 0.001$) in the number of endplates between the normal and denervated OOM (Fig. 6). In addition, a significant increase ($p < 0.001$) was observed in the number of endplates between the denervated and reinnervated groups (see Fig. 6). A comparison of the number of endplates between regions of the OOM (see Fig. 3), using a one-way ANOVA analysis, demonstrated a strong effect ($p < 0.001$; Fig. 7). Further analysis showed a significantly greater number of endplates in the lateral regions of the OOM ($p < 0.001$). Similarly, a comparison of the number of endplates per group of animals across regions of the OOM showed a significant interaction effect ($p < 0.001$). This relationship (see Fig. 7) was consistent across all groups of animals, with endplates showing a predominance (80 percent) in the lateral regions.

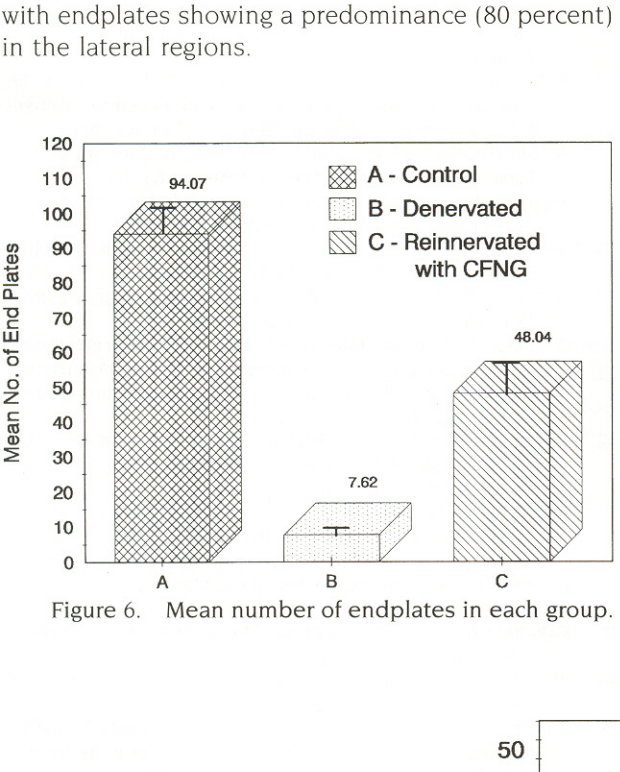

Figure 6. Mean number of endplates in each group.

DISCUSSION

The muscles that are responsible for facial expression and eye movements are among the most structurally and functionally diverse skeletal muscles. The eye-muscle fibers extend through the entire length of the muscle, are more rounded in transverse sections, and are smaller in diameter than most skeletal muscles. In addition, the ocular muscles, along with other facial muscles controlling fine movement, have the smallest number (< 10) of muscle fibers per motor unit,[14] while the larger skeletal limb muscles have a greater number of motor units (as high as 2000).

Previous studies have demonstrated that the OOM receives nerve input from the temporal and zygomatic branches of the VII nerve.[16] Unfortunately, less is known concerning the OOM pattern of innervation. Kakulas and Adams[15] described three patterns of skeletal muscle innervation: 1) The motor end-plates are scattered along the length of the muscle. 2) The end-plates are predominantly located at the ends of the muscle. 3) The motor end-plates are confined to the equator of the muscle.

In the present study, it was demonstrated that the motor end-plates of the OOM were confined to the lateral segments of the muscle. This would indicate a lateral entry of the motor nerve fibers into the rat OOM and this pattern of innervation has been observed by the senior author (JKT) in the majority of skeletal muscles harvested for free muscle transplantation procedures.

Reinnervation of the OOM with a CFNG retained this pattern of innervation. Also, animals treated with a CFNG showed a significantly greater number of motor endplates in the OOM, when compared to the denervated group. Thus, the CFNG-treated animals had significantly stronger efferent signals from the

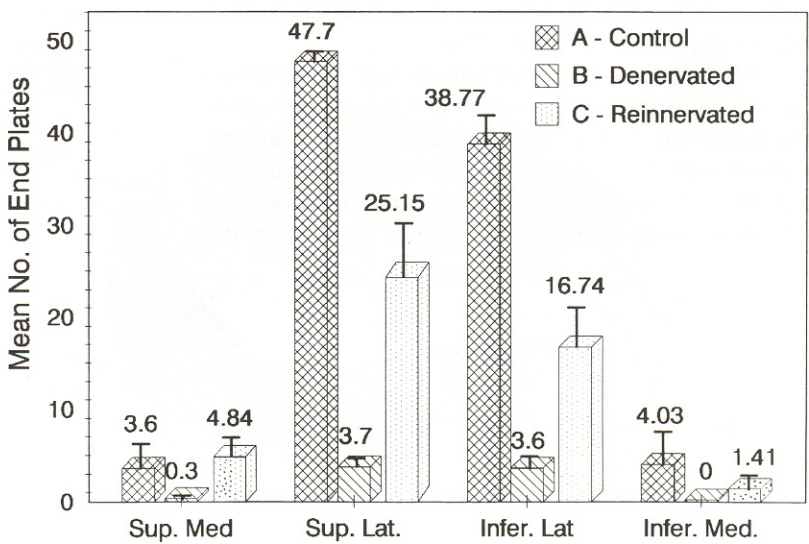

Figure 7. Mean number of endplates in each group per quadrant.

contralateral facial motor nucleus to the reinnervated OOM, resulting in coordinated eye closure and blink.

Porter and colleagues[17] examined the muscle typing of the primate OOM and the facial motor neurons that served it. This study reported that the primate OOM consisted of three distinct muscle fiber types. These were designated as slow-twitch, intermediate fast-twitch, and pale fast-twitch. The slow-twitch fiber type exhibited skeletal muscle fiber type I fiber-staining patterns, were smaller in diameter, and comprised about 10 percent of the total fiber number. In contrast, the intermediate fast-twitch and pale fast-twitch fibers exhibited skeletal muscle type II fiber-staining patterns, were larger in diameter, and comprised 90 percent of monkey OOM fibers. This predominance of the two fast-twitch fiber types in the OOM is consistent with the on/off function of the muscle in blinking. In addition, this study showed that the motor neurons serving the OOM were largely confined to the ipsilateral dorsal division of the facial nucleus.

The present study demonstrated that the utilization of a cross-facial nerve graft linking the paralyzed orbicularis oculi muscle to a contralateral normal upper zygomatic branch was able to generate a 50 percent increase in the number of endplates in reinnervated rats, compared to denervated animals. This was achieved by the technique of sharing motor axons microsurgically without sacrificing a functioning cranial nerve (for a donor) and its muscles, as was the case in some previous studies.[5,6] In addition, it was demonstrated that the motor endplates were apparent at 3 months post-reinnervation. This was in agreement with previous research on other muscles.[11–13]

The reported research presents a powerful experimental model to study the blink reflex in facial paralysis. Specifically, these data provide critical information on motor endplate quantification in the normal and denervated states, as well as in cases of restoration of eye sphincter function with cross-facial nerve grafts. This study strongly supports the development of different reconstructive strategies for target reinnervation and functional restoration.

Furthermore, it is well known that a significant number of facial paralysis cases involve patients seeking treatment 2 years or more after injury, thus presenting with largely atrophic orbicularis oculi muscles. In such cases, a nerve graft alone would not be an effective choice of treatment. Future research in our laboratory will be evaluating the provision of new autologous muscle to substitute for the denervated OOM. These studies will examine the quantitative and qualitative characteristics of a foreign target functioning as a new eye sphincter in animals with long-lasting paralysis. Finally, blink restoration in this model may be a possibility through the motor fibers of the contralateral facial nerve.

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